Production and Pro-Apoptotic Activity of Soluble CD95 Ligand in Pancreatic Carcinoma

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INTRODUCTION

CD95L (FasL) is a pro-apoptotic transmembrane protein whose extracellular domain can be released from the cell surface by proteolytic cleavage (1–4). Cell-associated CD95L induces apoptotic cell death by engaging its cognate receptor (CD95/Fas receptor/APO-1) on target cells, followed by recruitment and activation of intracellular enzymes of the caspase family, which initiate and execute the apoptotic process (5–7). High levels of CD95L expression have been observed in some hemopoietic (8) and solid malignancies, including hepatocellular (9), colonic (10), esophageal (11), and pancreatic (12, 13) carcinoma. In view of its potential in promoting apoptosis of immune cells, tumor-associated CD95L has been considered as part of the “counterattack” of tumor cells against immune effector cells. However, recent findings suggest more complex roles of the CD95L/CD95 system at the interface of tumor cells and the immune system. Some epithelial tumor cells have been found to secrete the proteolytically processed form of CD95L (14, 15), which has been reported to be a weak inducer of apoptosis compared with the membrane-associated form (16, 17). Furthermore, secretion of the ligand-binding domain of CD95 has been observed in certain tumor cells (18, 19). Soluble CD95 can act as a “decoy” receptor and competitively reduce binding of ambient CD95L to target cells (20). Thus, soluble CD95L may modulate the efficacy of cell-associated or soluble CD95L to induce target cell death. At present, the combined effects of secreted tumor-derived CD95L and CD95 isofoms on the survival of neighboring cells or the producing cells themselves are poorly understood.

In the present study, we investigated the relative contribution of secreted CD95L expressed by pancreatic carcinoma cells to the killing of target T cells in vitro. This was based on our observation of elevated levels of both CD95L and CD95 in the peripheral blood of pancreatic carcinoma patients. We demonstrate that CD95L secreted by pancreatic carcinoma cells in vitro contributed to killing of Jurkat T lymphocytes. Furthermore, pancreatic carcinoma cells were resistant to CD95-dependent induction of apoptosis when compared with Jurkat cells.

MATERIALS AND METHODS

Antibodies and Reagents. The following antibodies to CD95L/CD95 were used: polyclonal rabbit antiserum Q20 (Santa Cruz Biotechnology, Santa Cruz, CA) and MoAb clone 33 (Transduction Laboratories, Lexington, KY), which recognize an epitope within the intracellular domain of human CD95L; G247-4 MoAb (PharMingen, San Diego, CA), which recognizes...

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The abbreviations used are: CD95L, CD95 ligand; MoAb, monoclonal antibody; CM, conditioned medium; RT-PCR, reverse transcription-PCR; NK, natural killer.
both the membrane-bound and soluble forms of human CD95L; neutralizing anti-CD95L MoAbs NOK-1 and NOK-2 (Phar-Mingen; Ref. 14); anti-CD95 MoAb UB2 (Kamiya Biomedical Co, Thousand Oaks, CA); and agonist anti-CD95 MoAb CH-11 (Kamiya). Recombinant human IFN-γ was from R&D Systems (Abingdon, United Kingdom), and recombinant CD95L was from Upstate Biotechnology (Lake Placid, NY). The metalloproteinase inhibitor BB-3103 was a generous gift of British Biotech (Oxford, United Kingdom).

Patients and Tissue Samples. Twenty-two pancreas carcinoma patients (15 men and 7 women; ages, 39–71 years) who underwent surgical resections at the Medicosurgical Department, San Giovanni Battista e della Città di Torino, Molinette Hospital, Torino, Italy were studied. All patients were affected with histopathologically confirmed primary pancreatic duct adenocarcinomas representing stage II (n = 10), stage III (n = 2), and stage IV (n = 10) pancreatic neoplasms according to the classification by Warshaw and Fernandez-del Castillo (21). Pancreatic cancer tissue samples were frozen in liquid nitrogen immediately after surgical removal and prior to RNA extraction. Serum samples collected from patients prior to surgery and from five healthy donors were stored at −70°C until use.

Cell Lines and CM. Human pancreatic carcinoma cell lines Capan-2 (American Type Culture Collection, Rockville, MD), BxPC-3, and PT-45 (kindly provided by Dr. M. F. Di Renzo, Department of Biomedical Sciences and Human Oncology, University of Torino, Italy) were grown in DMEM supplemented with 10% FCS (Life Technologies, Inc., Grand Island, NY). The human acute lymphoid leukemia T-cell line Jurkat was cultured in RPMI 1640 (Life Technologies) supplemented with 10% FCS. All cell lines were routinely screened for coplasma contamination, using the Hoechst dye H33258 (Sigma). Recombinant human IFN-γ was from R&D Systems (Grand Island, NY). Human CD95L-specific primers were as follows: 5'-5'-CAC CTA CAG AAG G-3' and 3'-3'-9,5-CAG CTC TTC TGC CCC CAG G-3'. The PCR protocol was as follows: 36 cycles at 94°C for 1 min for denaturation, 55°C for 1 min for annealing, and 72°C for 1 min for extension. The predicted size of the CD95L PCR product was 447 bp. Human β-actin primers and amplification conditions were as described by us previously (23). PCR products were analyzed by size fractionation using 2% agarose gels stained with ethidium bromide.

Immunohistochemical Detection of CD95L. Immunohistochemical staining of formalin-fixed, paraffin-embedded tissues or cryosections was performed using anti-CD95L rabbit polyclonal antibody Q20 and the MoAb clone G247-4. Immunoreactivity was probed using the appropriate biotinylated secondary antibodies against rabbit and mouse immunoglobulins, as needed, and streptavidin-peroxidase complex (DAKO LSAB Peroxidase kit; DAKO, Carpinteria, CA). The reaction was visualized using the chromogen diaminobenzidine tetrahydrochloride with hydrogen peroxide as described previously (24). Counterstaining was performed with Mayer’s hematoxylin. To determine unspecific staining of Q20, the immunizing peptide that blocked polyclonal antibody binding provided by the manufacturer was used.

Immunofluorescence Staining and Flow Cytometry. Jurkat cells and pancreatic carcinoma cell lines, cultured for 24 h in the absence or presence of IFN-γ (1000 units/ml) and detached from plastic tissue culture flasks using 5 mM EDTA in PBS, were incubated with 1 μg of the anti-CD95 MoAb UB2 for 30 min at 4°C. Cells were washed twice and then incubated with FITC-conjugated (Fab)₂ goat antimouse IgG. After three washes, samples were analyzed on a FACScan (Becton Dickinson, San Jose, CA). A minimum of 10,000 events per sample were analyzed. Negative controls were carried out simultaneously using an isotype-matched MoAb with irrelevant specificity.

RT-PCR. Total RNA was extracted from BxPC-3, Capan-2, PT-45, and interleukin-2-activated NK cells derived from healthy donors as described previously (22). Pancrreatic adenocarcinoma tissue samples were extracted using the single-step RNAzol method (Cinna/Biotecx, Houston, TX). cDNA synthesis was performed at 37°C for 1 h using oligo(dT) primer in a final volume of 20 μl containing 20 units of Moloney murine leukemia virus reverse transcriptase, 1X reverse transcriptase buffer, 24 units of RNasin, and 0.5 mM deoxynucleotide triphosphate mixture. Ten μl of first-strand cDNA were added to 20 μl of PCR mixture containing 100 ng each of 5’ and 3’ primers and 1 unit of Taq DNA polymerase. All PCR reagents were purchased from Life Technologies (Grand Island, NY). Human CD95L-specific primers were as follows: 5’, 5’-CAG CTC TTC CAC CTA CAG AAG G-3’; and 3’, 5’-AAG ATT GAA CAC TGC CCC CAG G-3’. The PCR protocol was as follows: 36 cycles at 94°C for 1 min for denaturation, 55°C for 1 min for annealing, and 72°C for 1 min for extension. The predicted size of the CD95L PCR product was 447 bp. Human β-actin primers and amplification conditions were as described by us previously (23). PCR products were analyzed by size fractionation using 2% agarose gels stained with ethidium bromide.

Fig. 1 Calibration of an ELISA assay for detection of human soluble CD95L as described in “Materials and Methods.” A standard curve using serial dilutions of human recombinant CD95L in PBS containing 10% FCS is shown, demonstrating linear kinetics of detection at a concentration range between 2.5 and 200 pg/ml.
Assay for CD95L-induced Cell Apoptosis. Apoptosis of Jurkat target cells resulting from cocultivation with BxPC-3, Capan-2, and PT-45 as effectors was quantified by measuring target cell DNA fragmentation using the JAM test (25, 26). Briefly, Jurkat cells (5 × 10^4 cells/ml) were incubated with 10 μCi/ml [3H]thymidine (DuPont New England Nuclear, Boston, MA) for 5 h, washed three times with PBS, and resuspended in RPMI 1640 supplemented with 10% FCS. Aliquots (100 μl) of the suspension were cultivated in 96-well plates with or without 100 μl of the suspension of the pancreatic carcinoma cells (5 × 10^5 cells/ml) or in the presence of 100 μl of 5-fold concentrated CM derived from pancreatic carcinoma cells. To inhibit CD95L-dependent apoptosis, the pancreatic carcinoma cells or their CM were preincubated for 30 min at room temperature with 2.5 μg/ml anti-CD95L MoAb NOK-2 or an irrelevant isotype-matched MoAb. Treatment of labeled Jurkat cells, cultured alone, with 0.25 μg/ml of the agonist anti-CD95 MoAb CH-11 served as a positive control for CD95-mediated apoptosis. All cultures were incubated for 48 h at 37°C and cell-associated [3H]thymidine was determined using a β-scintillation counter. The reduction in incorporated radioactivity in experimental samples compared with untreated controls was used to calculate the percentage of target-specific killing [(cpm untreated cells – cpm cocultured cells/cpm untreated cells) × 100]. In addition to the JAM assays, acridine orange staining and fluorescence microscopy analysis were used to examine whether Jurkat cells exhibited nuclear changes characteristic of apoptosis after co-incubation with either the pancreatic carcinoma cells or their 5-fold concentrated CM.

Assay for Anti-Fas MoAb (CH-11)-induced Cell Death of Pancreatic Carcinoma Cells. Pancreatic carcinoma cell lines BxPC-3, Capan-2 and PT-45, treated for 24 h with or without IFN-γ (1000 units/ml) to induce CD95 expression, were labeled with 10 μCi/ml [3H]thymidine for 5 h, washed three times with PBS, and seeded in 96-well plates at 5 × 10^4 cells/well. To induce apoptosis, the agonist MoAb CH-11 was used. After 24, 48, and 72 h, cells were collected, and the percentage of target-specific killing was calculated as described above.

Immunoblotting. Immunoblotting of FasL protein contained in cell lysates was performed as described previously (27). Cells (2 × 10^6) were incubated for 30 min at 4°C in 200 μl of lysis buffer [50 mMol/L Tris-HCl (pH 8), 150 mMol/L NaCl, 2 mMol/L EDTA, 1 mMol/L EGTA (pH 7.5), supplemented with 1% Triton X-100 and containing 25 μg/ml aprotinin, 25 μg/ml leupeptin, and 1 mMol/L phenylmethylsulfonyl fluoride; all from Sigma], and cell detritus was pelleted by centrifugation at 13,000 rpm for 30 min at 4°C. Supernatants from serum-free cultures, either untreated or treated for 18 h with metalloprotease inhibitor BB-3103 (10 μM), were collected, concentrated 50-fold, and equilibrated with an equal volume of 2× lysis buffer. SDS-PAGE (12%) was performed using 30 μg of protein per lane under reducing conditions. An endothelial cell lysate, provided by Transduction Laboratories, was used as a positive control for cell-associated CD95L. After transfer to nitrocellulose membranes (Bio-Rad, Hercules, CA), the filters were blocked with 5% BSA in PBS containing 0.05% Tween 20 for 1 h at 4°C. The membrane containing cell-associated proteins was probed with anti-CD95L clone 33 MoAb (0.5 μg/ml), whereas the membrane containing soluble proteins in CM was probed using the G247-4 MoAb (1 μg/ml). After washing, membranes were incubated for 1 h with protein A-peroxidase conjugate (1:5000 dilution; Amersham, Arlington Heights, IL). After several washes, the blots were developed by chemiluminescence followed by autoradiography (Renaissance chemiluminescence reagent; New England Nuclear, Boston, MA). Densitometric evaluation of the radiographic signals was performed on digital images using Sigmagel software (Jandel, Heidelberg, Germany).

Statistical Analysis. To assess statistically significant differences between data sets, Student’s t tests for independent samples or Mann-Whitney rank-sum tests were performed using SigmaPlot (Jandel).

RESULTS

Soluble CD95L and CD95 in Sera of Pancreatic Carcinoma Patients. Secretion of both CD95 and CD95L by tumor cells has been implicated in modulation of the antitumor immune response. To assess tumor-associated production of soluble CD95 and CD95L in pancreatic carcinoma patients, we determined their levels in peripheral blood. We observed statis-
tically significantly elevated levels of both forms in sera from pancreatic cancer patients compared with healthy donors (Fig. 2). In both cases, the highest levels were observed in sera derived from patients afflicted with more advanced, i.e., inoperable, disease.

Production and Secretion of CD95L by Pancreatic Carcinoma Cells in Vivo and in Vitro. We next determined whether pancreatic carcinoma cells themselves were a potential source of either CD95 or CD95L in patients’ sera. Ungefroren et al. (12) recently described coexpression of CD95 and CD95L by pancreatic carcinoma cells in vivo and in vitro but found no evidence for shedding of CD95L by these cells. Consistent with these results, we observed expression of CD95L mRNA in pancreatic carcinoma tissue by RT-PCR (Fig. 3) and of CD95L protein by immunohistochemical staining, which revealed intense staining in the majority of carcinoma cells in situ (Fig. 4, 2451).
Similarly, we found CD95L protein in cellular extracts of three pancreatic carcinoma cell lines (BxPC-3, Capan-2, and PT-45). A, detection of cell-associated CD95L with an estimated molecular mass of 37 kDa as detected by MoAb clone 33; as positive controls, extracts from human endothelial cells were used. B, detection of a 24-kDa protein species in CM from pancreatic carcinoma cells, using an antibody that binds to an epitope in the extracellular domain of CD95L (G247-4). The metalloproteinase inhibitor BB-3103 was used to inhibit cleavage, and shedding of CD95L from cell surfaces as indicated; +, BB-3103 treatment; −, no treatment. C, reduction in signal intensity of BB-3103-treated pancreatic carcinoma cells relative to untreated controls as determined by densitometric analysis of the signals shown in B. Intensity is expressed in arbitrary pixel units.

A and B). Similarly, we found CD95L protein in cellular extracts of three pancreatic carcinoma cell lines tested (BxPC-3, Capan-2, and PT-45; Fig. 5A), which also expressed CD95L mRNA (Fig. 3) and secreted immunoreactive CD95L as determined by Western blot analysis of 50-fold concentrated CM (Fig. 5B). In all three assay systems (RT-PCR, immunoblot analysis, and ELISA) Capan-2 and BxPC-3 cells produced quantitatively similar high levels of cell-associated and shed CD95L, whereas PT-45 cells produced barely detectable CD95L mRNA and comparatively little protein. Specifically, as determined by ELISA of cell-free CM, Capan-2 cells secreted CD95L at a rate of 83.6 pg/ml/48 h, BxPC-3 cells at 75.2 pg/ml/48 h, and PT-45 cells at 19.6 pg/ml/48 h. Control medium not exposed to cells contained <5 pg/ml CD95L. Previous studies demonstrated that shedding of the transmembrane CD95L molecule requires cleaving of the extracellular domain by a metalloproteinase, resulting in a single band of apparent molecular mass 24–27 kDa when analyzed by PAGE (14, 16, 28). Consistent with proteolytic cleavage, the molecular mass of CD95L shed by pancreatic carcinoma cells was ~24 kDa, compared with 37 kDa for the cell-associated CD95L (Fig. 5).

We did not detect the 37-kDa molecular species in CM from pancreatic carcinoma cells, which argues against contamination of CM with cellular debris containing the transmembrane form of CD95L. In further support of the proteolytic release of CD95L from pancreatic carcinoma cells, accumulation of the 24-kDa protein species in CM from Capan-2, BxPC-3, and to a lesser extent, PT-45 was reduced by metalloproteinase inhibitor BB-3103 (Fig. 5, B and C), which recently was described to inhibit CD95L secretion in Ewing’s sarcoma cells (29).

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**Cytotoxic Effects of Pancreatic Carcinoma Cells on Jurkat Cells.** To assess the effect of transmembrane and shed tumor-derived CD95L on the survival of CD95L-sensitive target
cells, we determined the capacity of the pancreatic carcinoma cell lines under study to induce apoptosis of Jurkat T cells. This was done by performing direct JAM tests in which tumor and target cells were cocultured or by indirect JAM tests in which the effects of cell-free CM derived from pancreatic carcinoma cells on Jurkat cell survival were evaluated. Significant levels of Jurkat cell death ranging between 20 and 50% over background were observed in both experimental settings (Fig. 6). A neutralizing antibody to CD95L partially but significantly reduced the cytotoxic effects of pancreatic carcinoma cells and supernatants in direct and indirect JAM tests, respectively. Consistent with apoptotic death, acridine orange staining of Jurkat cells cocultured with Capan-2 cells revealed DNA condensation and nuclear fragmentation of Jurkat cells (Fig. 7).

**Pancreatic Carcinoma Cells Are Resistant to Apoptosis Induced by Secreted CD95L.** When cocultured with Jurkat cells, pancreatic tumor cells showed intact nuclei with no evidence of DNA condensation, suggesting resistance to the pro-apoptotic effects of CD95L shed by themselves (Fig. 7). This observation was confirmed for all three pancreatic carcinoma cell lines under study when JAM tests were performed using CH-11 to activate CD95 (results not shown). In some tumor systems, resistance to CD95L-mediated cell death has been found to be associated with down-regulation of CD95 expression on the cell surface (11, 30). We, thus, determined expression of CD95 in the pancreatic carcinoma cells under investigation. Compared with Jurkat cells, which expressed high levels of CD95 (88% of cells positive), all three pancreatic carcinoma cell lines expressed lower levels of CD95 ranging between 4 (PT-45) and 65% (Capan-2; Fig. 8). Treatment with recombinant IFN-γ (1000 units/ml) raised CD95 expression in all three tumor cell lines to various degrees. This effect was most pronounced in Capan-2 cells, 89% of which expressed CD95 under these experimental conditions, and least pronounced in PT-45 cells, 19% of which expressed CD95 in the presence of IFN-γ. However, IFN-γ-mediated up-regulation of CD95 did not result in significantly higher rates of either spontaneous or CH-11-induced apoptosis in pancreatic carcinoma cell lines as determined by JAM tests performed 24 and 48 h after IFN-γ treatment (not shown). After prolonged exposure to CH-11 for 72 h, only PT-45 cells showed a slight increase (20% over background) of IFN-γ-dependent apoptosis. By contrast, the agonist CH-11 very effectively induced apoptosis in Jurkat cells at 24, 48, and 72 h of incubation (>85%; also see Fig. 6).

**DISCUSSION**

This study demonstrates that progression of pancreatic carcinomas in patients is associated with increased serum levels of both the pro-apoptotic CD95L molecule and soluble forms of its receptor. Shedding of CD95L was also observed in pancreatic carcinoma cells in vitro, thus identifying one possible source of CD95L in patients’ sera. Elevated levels of soluble CD95L in patients’ sera have been reported earlier in NK cell lymphomas (8, 31). To our knowledge, this is the first report to demonstrate elevated serum levels of CD95L in patients with an epithelial malignancy. That malignant epithelial cells can secrete soluble CD95L was shown recently for prostate cancer cells in vitro (15). Our finding that pancreatic carcinoma cells similarly shed CD95L into their medium is at variance with the results of a recent study that described expression of cell-associated CD95L in pancreatic carcinoma cells in vitro but failed to detect soluble CD95L in CM of these cells (12). It is unclear whether this difference is due to the use of different cell lines and variants, different experimental conditions, or the sensitivity of the CD95L detection system used. However, it should be noted that two different groups detected a 26-kDa form of CD95L in cellular extracts of several pancreatic carcinoma cells, including BxPC-3, which is consistent with proteolytic processing, a prerequisite for shedding of CD95L (12, 13).

Elevated serum levels of soluble forms of CD95 have been reported previously in patients afflicted with hematoproliferative disorders (32), hepatocellular carcinoma (33), and bladder carcinomas (34). In bladder cancer, high serum levels of CD95 were associated with poor prognosis and shortened 5-year survival rates of the tumor patients. Our results suggest a similar relationship between CD95 serum levels and prognosis in pancreatic carcinoma patients because patients with more advanced tumors had significantly higher serum levels of CD95. At present it is unclear whether the tumor cells themselves contributed to this phenomenon in vivo because we found no soluble CD95 in media conditioned by the pancreatic carcinoma cells in vitro. Both secreted CD95L and CD95 previously have been implicated in inhibition of CD95-mediated apoptotic cell death. Soluble CD95 has been shown to act as a potential decoy receptor by competing with ambient CD95L for binding to a membrane-associated receptor capable of transducing death signals to the cell interior (18, 35, 36). Similarly, soluble CD95L has been shown in some studies to be a weak inducer of apoptotic death
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The nature and possibly synergistic actions of these factors in concert with CD95L are under investigation. In contrast to Jurkat cells, the pancreatic carcinoma cells themselves were resistant to the pro-apoptotic effects of CD95L. Partial or complete resistance of epithelial carcinoma cells to apoptosis induction by CD95L has been described previously and was traced in some cases to down-regulation of CD95 (11, 30). In the present study, resistance to CD95L could not be attributed to down-regulation of CD95 as demonstrated by lack of an apoptotic response to the pro-apoptotic CH-11 antibody even in those cells in which CD95 was up-regulated by IFN-γ to levels comparable to CH-11-sensitive Jurkat cells. These results confirm and extend the observations of von Bernstorff et al. (13), who similarly found reduced sensitivity of pancreatic carcinoma cells upon CD95 engagement even if CD95 expression was up-regulated by IFN-γ treatment. It seems possible that resistance of pancreatic carcinoma cells to CD95-triggered apoptosis is due, at least in part, to disruption of CD95-dependent signal transduction or the deregulated expression of intracellular inhibitors of apoptosis, including FLIPs (38), or FAP-1 (12, 39). Expression patterns of intracellular signal transducers and apoptosis inhibitors and their functional significance in apoptosis resistance of pancreatic carcinoma cells are under investigation.

Taken together, the results of the present study demonstrate that pancreatic carcinoma cells produce soluble CD95L that induces apoptosis in CD95-sensitive cells and could contribute to the demise of CD95L-sensitive target cells at a distance.

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REFERENCES


Fig. 8 Constitutive expression of CD95 by pancreatic carcinoma cells and induction by IFN-γ treatment. Results of FACS analysis using MoAb UB2 are shown in control media and in the presence of 1000 units/ml IFN-γ as indicated at the top. The denominations of the cell lines tested are shown on the right.

Compared with the transmembrane form of CD95L (8, 20). On the basis of these previous observations, we hypothesized that CD95L shed by pancreatic carcinoma cells should not induce apoptosis or even protect CD95L-sensitive target cells from CD95L-induced cell death. Contrary to this expectation, we observed that soluble CD95L contained in media conditioned by the three pancreatic carcinoma cell lines under study contributed to apoptosis of Jurkat cells. A substantial role of soluble CD95L in Jurkat cell death is supported by our observation that a neutralizing anti-CD95L antibody (NOK-2) significantly reduced the pro-apoptotic activity of CM derived from the three pancreatic carcinoma cell lines under study. This result is consistent with previous observations that soluble CD95L contained in CM from activated Jurkat cells contributes to target cell killing (37) and that CD95L shed by human prostatic carcinoma cells exerts pro-apoptotic activity (15). However, it is seemingly in contrast to the finding that Jurkat T cells are resistant to the pro-apoptotic effects of purified soluble CD95L (16). These discrepant observations can be reconciled by assuming that soluble CD95L added as a single factor to culture medium is a poor inducer of apoptosis in Jurkat cells, whereas efficient cell killing by soluble, pancreatic carcinoma-derived CD95L is contingent on cofactors contained in media conditioned by pancreatic carcinoma cells. Importantly, addition of neutralizing antibodies to pancreatic carcinoma CM significantly inhibited but did not block Jurkat cell death, which supports the notion that in addition to CD95L, other soluble factors contribute to the pro-apoptotic activity contained in pancreatic carcinoma CM. The nature and possibly synergistic actions of these factors in concert with CD95L are under investigation. In contrast to Jurkat cells, the pancreatic carcinoma cells themselves were resistant to the pro-apoptotic effects of CD95L. Partial or complete resistance of epithelial carcinoma cells to apoptosis induction by CD95L has been described previously and was traced in some cases to down-regulation of CD95 (11, 30). In the present study, resistance to CD95L could not be attributed to down-regulation of CD95 as demonstrated by lack of an apoptotic response to the pro-apoptotic CH-11 antibody even in those cells in which CD95 was up-regulated by IFN-γ to levels comparable to CH-11-sensitive Jurkat cells. These results confirm and extend the observations of von Bernstorff et al. (13), who similarly found reduced sensitivity of pancreatic carcinoma cells upon CD95 engagement even if CD95 expression was up-regulated by IFN-γ treatment. It seems possible that resistance of pancreatic carcinoma cells to CD95-triggered apoptosis is due, at least in part, to disruption of CD95-dependent signal transduction or the deregulated expression of intracellular inhibitors of apoptosis, including FLIPs (38), or FAP-1 (12, 39). Expression patterns of intracellular signal transducers and apoptosis inhibitors and their functional significance in apoptosis resistance of pancreatic carcinoma cells are under investigation.


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